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Septic thromboembolism in intravenous drug users
L. Kolilekas, M. Eliopoulou, G. Konstantopoulou, K. Loverdos, M. Gaga........ 51
A 70-year old female, lifetime non-smoker was admitted to our outpatient clinic complaining of mild productive cough, dyspnea on exertion and general fatigue. During the last ten years she reported multiple lower respiratory tract infections and was diagnosed with bronchiectasis based on compatible HRCT findings 5 years ago. Four years ago, *Pseudomonas Aeruginosa* was isolated from her sputum and was treated with oral ciprofloxacin for 21 days. During the last three years she reported no hospitalizations and was self-prescribing antibiotics during worsening of her symptoms.

On admission she was afebrile, thin and phtyic and had mild kyphoscoliosis. Her clinical examination revealed: SaO₂: 95%, (FiO₂: 21%), heart rate: 90 bpm, respiratory rate: 12/min, and inspiratory squeaks on auscultation, mainly localized on lower lobes. She had no clubbing or ankle edema. She reported no Raynaud’s phenomenon or other symptoms of arthritis (arthralgias, morning stiffness) or myositis. Her high resolution computed tomography revealed multiple cystic bronchiectases and nodular tree-in-bud opacities (Figure 1). A complete etiologic investigation of non-cystic...
fibrosis bronchiectasis was performed.

The patient was immunocompetent based on quantitative serum immunoglobulin and general blood tests, revealed no history compatible with pertussis infection at infancy or childhood and her serum immunologic profile was negative. She was HIV and hepatitis B and C negative. Her tuberculin skin test was 8 mm and the interferon-gamma release assay (IGRA-QuantiferonTB-gold) was negative. Her sputum smears (n=3), PCR assays and solid-medium (Lowenstein Jensen) culture were negative for mycobacterium tuberculosis (MTB). Solid medium cultures of sputum specimens revealed colonies of non-tuberculous mycobacterium avium complex (MAC). She was commenced treatment with a thrice-weekly regimen consisted of: rifampicin – 600 mg (qid), clarithromycin - 1000 mg (bid) and ethambutol-1000 mgr (qid). Two months later the patient reported significant improvement of her dyspnea, fatigue and cough as well as sputum purulence and volume.

The isolation of non-tuberculous mycobacterial (NTM) remains a clinical dilemma. Because NTM naturally exist in the environment, isolation of NTM from a non-sterile respiratory specimen does not necessary mean infection. NTM pulmonary infection develops commonly in structural lung disease such as chronic obstructive pulmonary disease (COPD), bronchiectasis, cystic fibrosis, pneumonoconiosis, prior tuberculosis, pulmonary alveolar proteinosis and esophageal motility disorders. In addition, clinicians should be highly aware and raise suspicion for NTM infection in cases of recurrent respiratory infections in immunocompetent individuals with radiological features of bronchiectasis.

More than 20 years ago, NTM pulmonary infection has been described in the context of Lady Windermere’s syndrome which typically consists of the phenotype of a thin, well-mannered elderly woman with voluntarily cough suppression, mainly middle-lobe bronchiectasis and pulmonary mycobacterium avium complex infection. The fastidious nature and reticence to expectorate are believed to be the main predisposing factors for lung infection by allowing secretions to collect into airways, particularly in the right middle lobe which has the longest and narrowest structure among lobar bronchi. The name originates from the lead character in Oscar Wilde’s play Lady Windermere’s Fan, which satirizes the strict morals and polite manners typical of the Victorian era in Great Britain.

The diagnosis of NTM pulmonary infection can be challenging and to this end clinicians should integrate atypical respiratory symptoms (cough, sputum, dyspnea) and radiological features (cylindrical bronchiectasis, multifocal tree-in bud opacities or cavitary lesions) to highly specific microbiological findings (positive culture for NTM in more than 2 expectorated sputum specimens or one specimen from bronchial lavage or washing) (Table 1). It is important to note that AFB stains (Kinyoun method seems to be superior to Ziehl-Neelsen) cannot distinguish between NTM and MTB. Nucleic acid amplification (NAA) tests are needed. Culture remains the gold standard for confirmation of NTM diagnosis. Culture media are similar to MTB. Both solid (Lowenstein Jensen) and liquid culture (Middlebrook 7H9) platforms are required. Nevertheless, since treatment and outcomes are different among NTM species, precise NTM identification is critical. Sequencing of the 16sRNA gene is the reference method of choice for NTM discrimination up to the subspecies level. Gene sequencing can also be used to identify Inducible macrolide resistance, especially in mycobacteria with rapidly growing taxonomy, such as M. abscessus complex.

Macrolides represent the cornerstone of NTM-MAC treatment (Table 1). Management can be difficult and lengthly (at least 12 months) and should be individually tailored based on the NTM species, disease symptoms, radiological extent and patients’ preferences. On the other hand, current guidelines suggest similar to MTB therapeutic regimens (except for pyrazinamide) for the treatment of M. kansasi, which is a relatively treatable pathogen. The therapy for M. abscessus still remains a bottleneck for physicians and researchers. Guidelines suggest an oral macrolide and two parenteral agents such as amikacin, imipenem, tygecycline, cefoxitin and linezolid for several months. Bedaquiline, tigecycline, linezolid and clofazimine (an anti-leprosy drug) represent therapeutic agents used for MDR-TB infections.

In NTM refractory cases, debulking surgery of the most affected area of the lung may be helpful in selected number of patients. In general, except from M. Kansasii, NTM infection is difficult to eradicate with anti-microbial therapy alone and is characterized by frequent relapses. Clinical trials enrolling patients with refractory NTM infection are sorely needed. Multiple combination therapies involving both surgical and anti-microbial interventions with novel therapeutic agents may hold promise for the future. Early referral to a reference center of excellence and multidisciplinary approaches are mandatory for optimal therapeutic decisions.
TABLE 1. Diagnostic Criteria and Therapeutic Approach for Non Tuberculous Mycobacterial (NTM)- MAC (Mycobacterium Avium complex) lung Disease

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<tr>
<th>Category</th>
<th>Criteria</th>
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<tr>
<td>Clinical</td>
<td>Pulmonary symptoms 1. Cough 2. Expectoration 3. Exclusion of alternative diagnoses</td>
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<tr>
<td>Radiological</td>
<td>1. CXR – Nodular or cavitary opacities 2. HRCT – Multifocal bronchiectatic lesions with multiple small nodules</td>
</tr>
<tr>
<td>Microbiologic</td>
<td>1. Positive culture in at least 2 sputum samples and AFB negative 2. Positive culture in at least 1 bronchial wash or lavage 3. TBB or other lung biopsy with granulomatous inflammation and positive culture for NTM and one positive culture in bronchial wash or lavage</td>
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<tr>
<td>Additional considerations</td>
<td>1. Clinical and radiological criteria are both required for diagnosis 2. Expert referral and consultation for diagnosis and treatment 3. Diagnosis does not necessitate treatment. Treatment should be individually tailored</td>
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Treatment

A. Daily regimen:
1. Macrolides (azithromycin 250 mgr or Clarithromycin 1000 mg/day)
2. Rifampin (rifampin or rifabutin) – 600 mg/day
3. Ethambutol 15 mg/kg/day

B. Thrice weekly regimen:
1. Macrolides (azithromycin 500 mg or Clarithromycin 1000 mg)
2. Rifampin 600 mg
3. Ethambutol 25 mg/kg

Duration
1. 18-24 months
2. At least 12 months after culture negativity

REFERENCES
Lung function in the elderly: Nascentes morimur

Panagiotis Panagou, Evangelos Bouros, Argyrios Tzouvelekis, Vassilios Tzilas, Demosthenes Bouros

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Key words:
- Lung function
- Pulmonary function
- Elderly
- Spirometry

Pulmonary structure and function change significantly between young adulthood and old age. Aging generates four important changes in the structure and function of the respiratory system. There is a reduction in the elastic recoil of the lung causing “senile emphysema”, a condition characterized by reduction in the alveolar surface area (elastic elements of the lung degenerate, parenchymal tissue is lost) without alveolar destruction, which is associated with hyperinflation, increased lung compliance and reduction in alveolar-capillary diffusing capacity. There is a decrease in the compliance of the chest wall, due to calcification of its articulations, dorsal kyphosis and “barrel chest”. There is a decrease in the strength of respiratory muscles (intercostal muscle mass and force are reduced) which correlates with cardiac Index, nutritional status and hyperinflation and there is a reduction in the ventilatory response to hypoxia and hypercapnia as well as in the perception of increased airway resistance.

Furthermore aging depresses cough reflexes and disturbances of innate immunity predispose the elderly to pulmonary inflammation. These changes affect pulmonary function tests and gas exchange, but adaptive changes in breathing frequency and tidal volume serve to maintain adequate ventilation and ventilatory responsiveness to hypoxia and hypercapnia.

Spirometry is underused and difficult to perform in older people and there is no spirometric gold standard specific in this population for the diagnosis of obstructive disease, with the most common error being the lack of a plateau at the end of exhalation, so a FET ≤6 s can be used. Imaging can to some extent integrate or also substitute for respiratory function data in highly problematic cases, providing important clinical information.

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop Summary has defined stage 1 chronic obstructive pulmonary disease (COPD) as airflow limitation where forced expiratory volume in one second/forced vital capacity (FEV1/FVC)% is ≤70% and FEV1% predicted is ≥80%. Stage 2 COPD has been defined as a FEV1/FVC% of ≤70% and an FEV1% pred of ≤80%. These criteria are set regardless of age in an attempt to simplify the diagnosis.

The trade-off with simplicity, however, comes at the expense of misclassification. Since the FEV1/FVC ratio falls with age, the use of a fixed cut-off point for defining COPD becomes more inaccurate with increasing age. These criteria were proven to lead to a significant degree of over-diagnosis of...
chronic airflow obstruction in those aged ≥70 yrs normal subjects and in those ≥80 years to even stage 2 COPD.8

Additionally one fifth of older adults with observed FEV1/FVC% above the NHANES-III fifth percentile had FEV1/FVC% ratios <70% (normals misidentified as abnormal).9

The lower limit of normal was estimated as: Predicted-1.65 x residual SD (i.e. the estimated 5th percentile). Furthermore in normal elderly blacks it was found that they had an FVC about 6% lower than elderly whites, even after correcting for standing height, sitting height (trunk length), and age, so the popular use of spirometry reference values from studies of middle-aged white subjects by applying a 12% race correction factor for black patients appears to overestimate predicted values.10

Also in another study cognitive impairment, shorter 6-min walk distance, and lower educational level were found to be independent risk factors for a poorer acceptability rate for spirometry (logistic regression analysis). Male sex and age were risk factors for a poorer reproducibility of FEV1, and reproducibility tended to improve with time.11

The use of Forced Oscillation technique (FOT) by impedance oscillometry (IOS) and in particular respiratory impedance (ZS), resonant frequency (Fres), and respiratory resistance (R5, R20, R5–R20) and respiratory reactance (XS) were shown to have good relevance compared with spirometry for geriatric patients, so IOS may serve as an alternative method for spirometry in elderly subjects for the evaluation of the state of lung function.12

Expiratory flow limitation (EFL) as assessed by the negative expiratory pressure method during tidal breathing may be also be of value in cases when spirometry is inadequate in the elderly.13

The calculation of spirometric Z-scores (predicted−measured/RSD) by Lambda-Mu-Sigma (LMS) rigorously accounts for age-related changes in lung function. Recently, the Global Lung Function Initiative (GLI)14 expanded the availability of LMS spirometric Z-scores to multiple ethnicities. Hence, in aging populations, the GLI provides an opportunity to rigorously evaluate ethnic differences in respiratory impairment. The LMS describes the mean (Mu) — representing how spirometric measures change based on predictor variables (age and height); the coefficient-of-variation (Sigma) — representing the spread of reference values; and skewness (Lambda, incorporating a spline function) — representing departure from normality. A Z-score of −1.64 defines the lower limit of normal as the 5th percentile of the distribution. Notably, using data from large reference populations of asymptomatic lifelong non-smokers, the GLI has recently published equations that expand the availability of LMS-calculated spirometric Z-scores, allowing respiratory impairment to be established across multiple ethnicities.

So by using these reference equations ethnic differences in an aging population was found in respiratory impairment, including prevalence and associations with health outcomes. In particular, African-Americans present a unique public health challenge, with high rates of respiratory impairment being associated with mortality but not respiratory symptoms.15

It was recently observed that a small proportion (7%) of subjects with CT-defined emphysema were identified by the 0.70 threshold for FEV1/FVC but not by the LLN. However, there is no evidence that CT-emphysema corresponds to a clinical entity that can benefit by inhaled therapy.16

In a cohort of very old adults, low FEV1 expressed as FEV1/HT3 was found to be a short-term predictor of all-cause mortality, hospitalization and decline in physical and mental functioning independently of age, smoking status, chronic lung disease and other co-morbidities. So FEV1/HT3 may be a potential risk marker for frailty and adverse health outcomes in the elderly.17

The incidence of airflow limitation per 1000 person-years was 28.2 using a fixed ratio and 11.7 with LLN, corresponding to a 1.41-fold higher incidence rate using a fixed ratio. The incidence increased dramatically with age when using a fixed ratio, but less so when using LLN. In addition, a sex effect was observed with the LLN criterion. LLN airflow limitation was associated with increased 5-year mortality. Presence of fixed-ratio airflow limitation in individuals classified by LLN as non-obstructive was not associated with increased mortality.18

In the Perspective of classic spirometry with MEFV curve was argued many years ago that it is an overall expression of the lung’s mechanical behaviour but reflects a very complex system and a series of mechanical events that is very poorly understood.19 So we come to the question: Do we need to measure airway resistance? Within the lung, at breathing frequencies, 50% of the resistance originates within the large airways, 40% within the lung tissue (due to dissipative frictional losses among the various structural elements), and only 10% within the small airways, again reflecting their enormous cross-sectional area. Because such a small amount of resistance emanates from the small airways, it is very difficult to detect changes in this area using conventional spirometry, and so this region has
been dubbed the “Silent” or “Quiet” zone of the lung. Because of this RAW is more sensitive than spirometry to detect changes in the aging lung.

Since Raw is highly dependent on lung volume, it is better expressed as specific airway conductance, sGaw, where sGaw = (1/Raw)/ thoracic gas volume (TGV). sGaw is a measure of intrinsic airway resistance, which is volume independent.

Raw can also be measured by the interrupter technique (Rint), and the forced oscillation technique (RFOT), both of which are performed during quiet breathing and require no special maneuvers like the FEV1. As such, both Rint and R-FOT are also more sensitive indicators of intrinsic Raw than FEV1.

In addition, the FOT offers additional insight into the elastic properties of the respiratory system and airway distensibility as well as into the homogeneity of ventilation. Because these methods are non-invasive and can be performed during quiet breathing, they have special appeal for patients who cannot perform spirometry or may have difficulty with proper technique, including children, the elderly, patients during sleep, or those with neuromuscular disease. Each of the methods has its own advantages and disadvantages.

In conclusion: 1) GLI reference equations for spirometry should be used by all lung function laboratories for all ages and ethnic groups. 2) The GLI LLN 5th percentile may be used along with GOLD guidelines to detect changes in lung function in the elderly in order to avoid overdiagnosis of airway obstruction. 3) In cases of clinical doubt more sensitive RAW measurements may have a role.

REFERENCES

Introduction of new technologies in Pneumonology training
Medical students can show us the way

Eleni Pilitsi¹, Paschalis Steiropoulos²

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SUMMARY
BACKGROUND: Over the last years new technologies are used to enhance the quality of medical education and to improve the educational experience. Social media are becoming popular tools for augmenting the effectiveness of education in various academic aspects and, especially, YouTube has been used as an adjunctive tool in medical students’ training. Aim of the study was to examine the use of YouTube videos as learning tools for Pneumonology clerkship by the students and their impact in the acquisition of clinical skills and theoretical knowledge. METHODS: An anonymous, online survey completed by medical students attending their fourth year of studies at the Medical School, Democritus University of Thrace (six year curriculum) was conducted. The questionnaire contained demographic questions and questions about the potential benefit of YouTube channel videos in the pulmonary training of medical students. RESULTS: Response rate was 87%. Respondents’ perception was that YouTube channels are useful as educational tools. Specifically, 41.1% of them reported getting “very much” or “much” benefit from online videos and the percentage increased to 65%, when specific videos were used as examples (p<0.001). CONCLUSIONS: Usage of YouTube videos as adjunct educational tools has an apparent positive impact on students’ comprehension of Pneumonology. Therefore, their value as a potential official training method should be further tested and could be strongly considered in the future. Pneumon 2018, 31(1):17-23.

INTRODUCTION

Over the last years, various new technologies have emerged and have become available to most Medical Schools in developed countries, for the
E-learning, simulation based techniques, virtual reality, interactive 3D software, educational video games, podcast/vodcast and social media including Facebook, Twitter, WhatsApp and YouTube, are all used in order to increase the quality of medical education offered and to improve the educational experience of medical students. These technologies have introduced a whole new era into medical education, addressing to the rapidly expanding knowledge.

However, not all universities have the financial capability to experiment with the previously mentioned technologies, nor they can afford the burden of incorporating those in their curriculum. Greece is one of the less privileged countries, due to the ongoing financial crisis that started in 2010. As expected, Greek Medical Schools were not able to make significant changes in the traditional ways of training young doctors. It is obvious that the necessity of curriculum modernization appears imperative; however it is conflicting with the lack of funding to the Greek Universities. Thus, less expensive methods to improve the quality of Greek medical education need to be used, one of which might be educational YouTube channels.

Social media are becoming very popular tools for enhancing the effectiveness of education in diverse academic areas. YouTube, a web-platform that enables users to watch, post, share, like/dislike and discuss videos, is the third most frequently used social media worldwide with more than 1 billion users, according to statistics available at YouTube's site. There is already some literature regarding the use of YouTube channels as adjunctive tools in training medical students and residents in specific fields. YouTube channels contain videos aiming at improving technical skills as well as at acquiring and retaining theoretical knowledge. There are studies that have already evaluated the use and effectiveness of social media, such as Twitter, in various clerkships/courses and show emerging positive results.

Anatomy, for instance, is one of the courses that can significantly benefit from YouTube videos due to the complexity of understanding 3D structures and organ relationships from printed or digital atlases. Jaffar concluded that YouTube can be considered as an effective educational tool and that faculties should produce their own videos to encourage student participation.

As per November 2017, there is no published data describing or assessing the methods used by Greek medical students in understanding Pneumonology. Thus, we aimed to record how often students in our Institution are using YouTube videos as a learning tool for their Clerkship in Pneumonology; which YouTube channels they mostly prefer, and which is the perceived effect of these videos in both clinical skills and theoretical knowledge acquisition.

METHODS

We conducted an anonymous, online survey among medical students attending their fourth year of studies at Medical School of the Democritus University of Thrace taking a Pneumonology course. The questionnaire addressed 16 questions: certain ones were of demographic type, some involved the general medical education experience so far and others were concerned with the potential benefit of viewing YouTube channel videos in the Pulmonary training of medical students.

More specifically, the general questions were referring to gender and age information. In addition, students were asked to report the number of courses not yet taken from previous semesters along with frequency of attending lectures. Furthermore, we specifically asked the students about subject matter in the Pneumonology posing conceptual difficulty and about certain weak parts in their physical examination skills that lead them to search online for explanatory YouTube videos. They were also asked to report the frequency of searching online videos, the impact of these videos and the effectiveness that a specific YouTube channel, may have on their understanding of the material. In order to capture the frequency/intensity of responding to the above questions, we used a quantitative scale from 1 to 5, with 1 representing “never/not at all” and 5 “always/very much”.

RESULTS

The survey was anonymously answered by 114 (from a total number of 130, response rate 87%) medical students attending the Pneumonology class during the second semester of the academic year 2016-1017. About half of the participants (50.9%) were female, aged between 21 and 26 years, with the vast majority being younger than 24 years old. Age distribution was as follows: 21 years (19.3%), 22 years (33.3%), 23 years (35.1%), 24 years (3.5%), 25 years (5.3%), and 26 years (3.5%).

The number of previous semester courses that students needed to take again ranged between 0 and 17. The median was 2 courses.

Frequency of lecture attendance by the students
was as follows: 21.1% (n=24) of them reported “always attending”, 40.4% (n=46) “frequently attending”, 26.3% (n=30) declared “sometimes attending”, 10.5% (n=12) “rarely attending” and 1.8% (n=2) “never attending”, as demonstrated in Figure 1.

An attempt was made to investigate possible association between the reported lecture attendance frequency and the number of courses that need to be retaken. More specifically, the objective was to discover whether students attending lectures at a low frequency (response ranging between never and sometimes), need to retake more courses compared to students attending classes at a high frequency (response ranging between frequently and always); however, no such association was revealed. Nevertheless, students attending lectures at the highest frequency appeared to have retaken very few courses.

According to students’ replies, the most difficult parts of the Pulmonary course are considered to be respiratory physiology and pathophysiology, acid base disorders, interstitial lung diseases and lung cancer. Additionally, students reported difficulties in reading chest CT scans. However, the latter particular skill should mostly be developed during the Radiology course.

Our students were also asked to rate the level of comprehension of Pneumonology-related material via the existing educational methods. The responses are shown in Figure 2.

We used a scale from 1 (very poor) to 5 (excellent). Data suggest that our students do not gain the maximum out of their Clerkship, since there is undoubtedly room for improvement.

The majority of students (87.7%) search online for videos related to the Pneumonology course in order to better comprehend the material and prepare for the final exam at the end of the semester. Four students (3.5%) reported doing this every time they study, 24 students (21.1%) almost always, 40 students (35.1%) frequently, 32 students (28.1%) rarely and 14 students (12.3%) never.

In order to qualitatively assess whether watching medical videos on YouTube has already helped our students comprehend difficult chapters and concepts in Pneumonology we used the scale from 1 (not at all) to 5 (very much). More specifically, 18 students (16%) stated that they were benefiting very much, 28 students (25%) much, 46 students (41%) fairly, 12 students (11%) slightly and 8 students (7%) not at all.

In order to assess whether lecture attendance frequency correlates to students’ response to the questions about effectiveness of YouTube videos in studying during Pneumonology rotation and preparing for the exam, we divided the students into two groups. The first group consisted of students who attended lectures at a high frequency (answers given: very frequently or always) and the second group consisted of students attending lectures at a low frequency (answers given: never, rarely or sometimes).

Out of the 70 students with increased lecture attendance frequency, 36 answered with high scores (4 or 5) in the video effectiveness question as well. From the 44 students who reported low lecture attendance, only 10 ranked high (with 4 or 5) the potential effectiveness of YouTube videos. In other words, 51% of students attending lectures with high frequency believe that YouTube videos can help them comprehend Pneumonology bet-
ter, whereas only 23% of students not attending lectures have similar point of view.

An attempt to explore a possible association between the number of courses needed to be repeated and the perceived effectiveness of YouTube videos revealed the lack of any association. Specifically, the number of courses students needed to reiterate was independent of their perception of YouTube effectiveness. No significant difference was determined in perceived YouTube effectiveness between males and females participating in our study.

Additionally, we collected more information regarding the most popular YouTube channels among students from our Institution. Only 80 of the students replied to this specific question. Fifty two students did not mention using any specific YouTube channel. Among the rest of the responders (28), Osmosis was the most popular channel, followed by Dr. Najeeb lectures and Armando Hasudungan channel.

To further investigate the value of YouTube videos as adjunct learning tools we proposed a series of Pneumonology related videos by Osmosis YouTube channel, which is becoming more and more popular among medical students worldwide, including Greek medical students, (367,524 subscribers and 22,442,849 views, as per November 28, 2017, according to numbers presented at Osmosis YouTube channel). Students were asked to watch these videos and comment on their effectiveness in order to understand Pneumonology related topics more efficiently. Our findings are illustrated in Figure 3, which contains the rating of YouTube videos effectiveness up until students were exposed to Osmosis videos compared to the rating after watching these videos.

There was an increase in the number of respondents ranking the effectiveness of Osmosis videos with either 4 or 5 compared to the general question about the effectiveness of online videos in the learning process. More specifically, 36 of the students having ranked the potential effect of YouTube videos with an 1 or 2 or 3, changed their mind when they watched the Osmosis videos by choosing 4 or 5 (8 students of those that picked 2, changed to 4, 22 students of those that picked 3, changed to 4 and 6 of those that picked 3, changed to 5).

Moreover, the average rating of the perceived effectiveness of YouTube channels before watching Osmosis videos was 3.32, whereas after watching Osmosis videos the average increased to 3.77. Paired sample t-test examination revealed that this increase in average rating before and after watching Osmosis videos was statistically significant (p<0.001).

An additional finding was that medical students in our Institution refer to YouTube videos for enhancing their clinical skills required in Pneumonology and their knowledge about routinely used interventions in clinical practice. We specifically asked the students about certain parts of physical examination and some of the most frequently used interventions in clinical medicine. Our findings are summarized in Table 1.

Lastly, we inquired about any perceived improvement in the level of technology incorporation into the medical curriculum and medical education, since the beginning of the medical studies of our study group, four years ago. Our students' perspective is presented in Figure 4.

**DISCUSSION**

The results of our study contain valuable messages that should be carefully interpreted. One of the most

<table>
<thead>
<tr>
<th>Clinical skills</th>
<th>Number of students</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>History taking</td>
<td>10</td>
<td>8.8</td>
</tr>
<tr>
<td>Lung palpation</td>
<td>20</td>
<td>17.5</td>
</tr>
<tr>
<td>Lung percussion</td>
<td>28</td>
<td>24.6</td>
</tr>
<tr>
<td>Lung auscultitation</td>
<td>84</td>
<td>73.7</td>
</tr>
<tr>
<td>Venous blood sampling</td>
<td>30</td>
<td>26.3</td>
</tr>
<tr>
<td>Arterial blood gas sampling</td>
<td>44</td>
<td>38.6</td>
</tr>
<tr>
<td>Placement of thoracic drainage</td>
<td>30</td>
<td>26.3</td>
</tr>
</tbody>
</table>

**FIGURE 3.** Perceived effectiveness of YouTube channels before and after watching the Osmosis videos in comprehending difficult concepts (1 for not at all, 5 for very much).
important findings is the feeling of our students that they have accomplished only an intermediate level (3 in a scale from 1 to 5) of comprehension of pneumology course with the existing teaching methods. The above finding may have various etiologies, but it is obvious that there is room for improvement regarding the educational experience offered.

One of the many ways to upgrade medical education that has been proven effective even since the 80s is to assimilate new media and technologies into traditional teaching.

YouTube hosts a great variety of educational channels with numerous medical topics accessible by medical students and graduates all over the world, provided that there is an internet connection. Most channels offer at least part of their video collection at no cost, so they constitute a good solution for all those having a limited budget. There are many different kinds of videos offered, some contain graphics and text, others animation, others recorded lectures or drawings. Thus, medical information can be taught in a more creative and understandable way compared to traditional teaching methods. Additionally, incorporation of social media, and, particularly YouTube videos, in medical education can trigger students’ interest about the topic, motivate them to engage more actively in the learning process and keep them concentrated for longer periods. However, we should not ignore the fact that the quality of these videos varies, depending, among other factors, on the source. Thus, not all channels should be blindly trusted. More specifically, there are three types of videos hosted on YouTube:

- Videos uploaded by individuals that are not part of an official educational process
- Videos uploaded by channels created to provide education such as official Medical School channels, Medical Organizations, Health care related companies
- Videos uploaded by channels related to educational sites. An example under this particular category is FOAMed (Free Open Access Medical education), a network of free, online educational services and resources, such as blogs, podcasts, tweets, Google hangouts, online videos, text documents, photographs, Facebook groups and many more. The main goal is to provide means to enhance traditional education. It is self-sponsored or supported by advertisements and does not require a registration fee from the user. Osmosis videos belong to the same category, since they are linked to a website that offers educational material, mnemonics, quizzes, question banks and reference articles. The videos are part of the open access content, but there is also the option to purchase a plan after a free trial.

Furthermore, information shared on the Internet is not strictly regulated, which is a major drawback when considering the possibility of using social media in medical education and learning. Apart from this, policies about the professional use of social media need to be clearly set and taught by the Institution, given the continuous alterations in the social media environment.

It is common practice that medical students utilize social media such as YouTube videos to obtain medical knowledge and cultivate their current skills, but there is limited amount of literature reporting this attitude and evaluating the impact on medical students’ performance. According to the results of the present survey, we concluded that the utilization of YouTube videos may help medical students obtain a better understanding of concepts taught in the pneumology course, especially if the videos are oriented toward course objectives. However, it is obvious that quality and content of the videos of each YouTube channel influence their impact on the learning experience.

According to our findings, there is a clear difference in perception regarding effectiveness of YouTube videos in studying during pneumology rotation and in exam preparing among students that regularly attend lectures compared to those that do not. This fact may have various possible explanations and needs further investigation.
Furthermore, the aforementioned finding should be assessed taking into account a study from the literature suggesting that students’ opinions about the efficiency of social media as learning tools vary between preclinical and clinical years. Particularly, students’ perspective may be dependent upon the academic year attending\(^4\).

To conclude, utilization of social media, like YouTube, in medical education is very promising in terms of enhancing the learning and educational experience, but certain aspects of this new trend need further investigation. In our study, which comprised a relatively small sample of medical students, Osmosis channel appears to be more helpful and effective in self-learning than other channels. All these findings should be taken into account by Administrators of Medical Schools in Greece, which have been in the process of reforming the curricula in an attempt to approach a more technologically up to date level, while facing continual financial problems that considerably influence the available solutions.

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2002; 77:925.
Biomedical Applications of Biopolymers in Airway Disease

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Key words:
- Polyhydroxyalkanoates (PHAs)
- Lung disease
- Lung tissue engineering
- Drug delivery systems
- Nanovaccinology

INTRODUCTION
The term biopolymer includes high molecular weight polymeric structured produced by living organisms with biological methods as opposed to synthetic polymers that are produced by chemical methods. Biodegrad-
able biopolymers have gained a great deal of scientific and industrial interest because they can be produced by a wide range of sources and be used in a growing range of biomedical applications. The organic bioplastics, i.e. biopolymers, are derived from renewable biomass sources such as vegetable oils, starch, proteins, etc., as opposed to petroleum-derived fossil fuels. Biopolymers provide the dual benefits of conserving mineral resources and reducing CO₂ emissions, which make them an important innovation for sustainable development.

BIOSYNTHESIS OF POLYMERS

Polymers

Biodegradable polyesters providing a sustainable alternative to petroleum-originated plastics consist of ester, amide and other functional groups that can be categorized into four classes, based on their synthesis process: i) natural polymers of plants and animals origin e.g. cellulose, chitosan, starch, and proteins, ii) microbial biopolymers like polyhydroxyalkanoates (PHAs), iii) polymers synthesized from natural monomers like polylactic acid (PLA), and iv) conventional polymers chemically synthesized from monomers produced from petrochemical products e.g. polycaprolactone. Additionally, the properties of these biodegradable polymers are usually altered and improved through blending. The potential sources for their biosynthesis varies from different sorts of biomass, including proteins, lipids and polysaccharides (such as cellulose- and starch-based biopolymers, chitosan) (Figure 1).

Proteins

In this category of biopolymers, the proteins that often are used are albumin, casein, collagen, feather meal (by product of poultry processing), gelatin, gluten, meal soy, peanuts, whey, and zein (a class of prolamine protein found in corn). Collagen is a naturally occurring structural extra-cellular matrix polymer and the predominant component of the mammalian body connective tissue, which is highly conserved across species. Biopolymers synthesized by collagen are often the best candidates for synthetic replacement of connective tissues due to their excellent structural and mechanical properties. Collagen biomedical applications in regenerative medicine are described in detail elsewhere.

A gelatinous protein mixture used for many applications and known with the commercial name matrigel is secreted by Engelbreth-Holm-Swarm mouse sarcoma cells, produced and commercialized by Corning Life Sciences and BD Biosciences. Matrigel is utilized by cell biologists as a matrix for cell culturing due to its resemblance to the complex extracellular environment that lies in various tissues. Gel foam is another gelatin-derived biomaterial that is used as an efficient hemostatic agent during surgical procedures.

FIGURE 1. Biosynthesis of polymers that are used to treat airway diseases.
Polysaccharides

Chitosan is a natural polysaccharide, with cationic and biocompatible properties constituted of co-monomeric units, 2-deoxy-2-acetamido-D-glucose and 2-deoxy-2-amino-D-glucose. The major advantage of chitosan is its mild antimicrobial activity that is attributed to its cationic residue, making it an important biomaterial since it suppresses bacterial growth by adhering to the bacterial cell wall. Furthermore, chitosan is biocompatible with human tissues and biodegrades in vivo. Its functional groups (hydroxyl, amine and amide) can be chemically modified to synthesize polyhydroxyalkanoates/chitosan mixtures that are applicable in wide range of biomedical applications.

Microbial polymers

Polyhydroxyalkanoates (PHAs) belong to a family of microbial polyesters and constitute the only bioplastics, synthesized by several Gram-negative and Gram-positive bacteria. PHAs serve as both source of energy for bacterial cultures and carbon storage. We have shown that PHAs can be synthesized in Thermus thermophilus under nutrient starvation conditions. PHAs can be combined with more than 150 different monomers and give rise to a wide range of biomaterials with various properties making them ideal candidates for a number of biomedical applications. Depending on their chemical structure, PHAs display flexible mechanical, structural, and thermal properties, biodegradability, biocompatibility and they are environmentally friendly. PHAs are often used in medicine as biodegradable and biocompatible implants and drug delivery capsules.

Poly-α-hydroxy acids

The most well-known poly-α-hydroxy acid is polyglycolideor poly(glycolic acid) (PGA). It constitutes the simplest linear, aliphatic polyester that is ranked among the biodegradable, thermoplastic polymers. Its biosynthesis takes place through polycondensation or ring-opening polymerization of the smallest α-hydroxy acid (AHA), or by solid-state polycondensation of halogenoacetates. Initially PGA had very limited use due to its tough fiber-forming structure and its rapid hydrolysis rate compared to other polymers. However, when PGA is coated with L-lysine and N-laurin, it makes an ideal soft bio-absorbable material for sub- and intra- cutaneous sutures and closures, respectively, in abdominal and thoracic surgeries. In the past decades PGA has been co-polymerized with a number of other different monomers such as lactic acid, trimethylene carbonate, e-caprolactone to bioengineer implantable medical devices including anastomosis rings, pins, rods, plates and screws.

Table 1 summarizes the biosynthesis and current applications of biopolymers used in airway diseases.

APPLICATIONS IN AIRWAY DISEASE

Lung Tissue Engineering

Engineering of lung tissue is part of the regenerative medicine that aims to reconstruct tissue parts and repair physiological functions of the lung rendered dysfunctional after lung injury or lung disease. Although there has been some progress in the de novo lung tissue engineering and transplantation of live human cells into patients to confront several respiratory diseases, it is not yet a clinical reality. Considerable effort has been placed to design matrices that can support 3-D structure, lung cell differentiation, and tissue development. Biopolymers such as collagen, gel foam and matrigel have been employed in lung tissue engineering and have been shown to allow lung tissue growth, albeit the development of a whole functioning organ has not been substantiated so far.

The biomaterials used for these purposes are expected to be biocompatible and their adsorption kinetics must be such so that the biopolymers will remain long enough to allow cell colonization and differentiation, without impeding the mechanical properties of the bioengineered tissue. It is now realized that the complexity of the human lung cannot be mimicked by a single biomaterial and development of a hybrid of biopolymers is required to generate lung tissue and different pulmonary cell types that can replicate the specific functions of the lung. For example, Club cells (Clara cells) that are found in the lung bronchioles, the function of which is to protect the bronchial epithelium, have been shown to differentiate from mouse embryonic stem cells on several biopolymers such as gelatine, collagen types I, IV, and VI either in submerged or air-liquid interface cultures. Another example is the alveolar type II pneumocytes; these produce the pulmonary surfactant that has critical role in reducing the surface tension formed at the air-liquid interface of the alveoli. We have shown that type II cells can maintain their phenotype in vitro in 3-D cultures system when grown on mixture of matrigel and collagen. We have also shown that upper airway nasal epithelial cells maintain their ciliated phenotype when grown in vitro in collagen IV coated air-liquid surfaces.
Lung cancer is by far the commonest form of cancer worldwide, with 1.7 million new cases just in 2012, a 13% annual incidence, and a leading cause of cancer death among both sexes. It is estimated that more people die of lung cancer than breast, prostate and colon cancers combined.24 Surgery and radiotherapy are the most common methods to remove and treat local, non-metastatic malignancies, while chemotherapy is employed to treat the metastatic cases of lung cancer. One of the major drawbacks of chemotherapy is that although the anti-cancer drugs are designed to target the fast dividing cells, they are not highly specific for just cancer cells, and often this lack of selectivity results in damage of healthy cells and adverse side effects. Furthermore, the half-life of these anti-lung cancer drugs is very transient in the blood stream, with low efficacies, and therefore higher doses of chemicals are needed with concomitant dire side effects.

In this sense, customized bio-absorbable nanostructured drug delivery systems (DDS) can offer great breakthroughs in the fight against lung cancer.

**Bio-absorbable Nanostructured Drug Delivery Systems**

DDS have a wide range of advantages compared to regular chemotherapy. Not only they can deliver anti-cancer agents in a controlled time and release rate but they can be customized to target lung specific cells and tissues and maintain efficient therapeutic drug levels.25 Polymeric DDS can be bioengineered in different forms.

### TABLE 1. Biopolymers used in airway disease, their origin and their biosynthesis.

<table>
<thead>
<tr>
<th>Biopolymers</th>
<th>Application</th>
<th>Origin</th>
<th>Biosynthesis References</th>
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<td>Chitosan</td>
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<tr>
<td>Collagen types i, iv, and vi</td>
<td>Cartilage graft, 3-D cultures system</td>
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<td>DODAC:DOPE (dioleoyl-dimethyl-ammonium chloride: dioleoyl-phosphatidyl-ethanolamine)</td>
<td>Nanobeads</td>
<td>Liposomes</td>
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</tr>
<tr>
<td>Gel foam</td>
<td>Lung tissue engineering, hemostatic agent</td>
<td>Porcine skin gelatin</td>
<td>65</td>
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<tr>
<td>Gelatine</td>
<td>Lung tissue engineering</td>
<td><em>Acetobacter xylinum</em></td>
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<td>HYAFF-11</td>
<td>Nasal Cartilage Graft</td>
<td><em>Streptococcus zooepidemicus</em></td>
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<td>Matrigel</td>
<td><em>In vitro</em> airway cell culture</td>
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<td>Polyethylene glycol (PEG)-substituted polylysine/PEBP-b-PBYP-g-PEG</td>
<td>Nanostructured drug delivery systems</td>
<td>Chemical agent</td>
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<td>Poly-amino acids</td>
<td>Nanoparticles</td>
<td>Plants</td>
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<tr>
<td>Polyethylene imine (PEI)</td>
<td>Nanovaccinology</td>
<td>Chemical agent</td>
<td>70</td>
</tr>
<tr>
<td>Poly-hydroxy alkanoic acids (PHAs)</td>
<td>Nanoparticles</td>
<td>Plants, <em>Thermus thermophilus</em></td>
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<tr>
<td>Poly-lactic-co-glycolic acid (PLGA)</td>
<td>Cartilage graft engineering, Nanovaccinology</td>
<td>Chemical agent</td>
<td>71</td>
</tr>
<tr>
<td>Polylysine/glycocylated polylysine and polyethylenimine</td>
<td>Nanoparticles</td>
<td><em>Streptomyces albulus</em></td>
<td>72</td>
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<tr>
<td>Polysaccharides</td>
<td>Nanovaccinology</td>
<td><em>Leuconostoc mesenteroides</em>, starch</td>
<td>73</td>
</tr>
<tr>
<td>Poly-α-hydroxy acids</td>
<td>Nanoparticles</td>
<td>Chemical agent</td>
<td>74</td>
</tr>
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</table>
(liposomes, micelles, micro- and nano-particles) infused with the appropriate anti-lung cancer agent and administered in different routes such as oral (inhaled DDS), injectable gels (blood stream DDS) and surgical implants (DDS scaffolds, foams, films/sheets). An additional feature of the bio-absorbable DDS is that after they deliver the desired anti-cancer agents, the biopolymers themselves can be metabolized by the patient’s body.

An example of increased efficacy of biopolymers is the PLGA nanoparticles loaded with the anti-lung cancer agent suberylanilidehydroxamic acid (SAHA). It was shown in vitro that these particles were able to release an initial burst of SAHA followed by sustained release for up to 50 h, showing higher antineoplastic activity compared to direct SAHA administration in human adenosacrinomic alveolar basal epithelial A549 cells. Another example of utilization of biopolymers to increase specificity in lung cancer cells is the bioengineering of PLGA nanoparticles coated with vascular endothelial growth factor receptor (VEGFR) on their outside surface and their infusion with paclitaxel, a tubulin-binding agent, which is widely used for the treatment of non-small cell lung cancer. The concept is that since vascular endothelial growth factor is over expressed in lung cancer cells, the coating of the nanoparticles with the receptor (VEGFR) facilitates the specific conjugation of the nanoparticles to the cancer cells and subsequent increased inhibitory activity of tumor growth compared to native paclitaxel or paclitaxel-loaded PLGA nanoparticles in the A549 cell line. Additionally, in vivo mouse studies showed that biopolymeric DDS can be used to prevent lung cancer metastasis to other organs. Yang et al. identified a peptide that specifically binds to pulmonary adenocarcinoma tissue, and conjugated it to PLA particles encapsulated with anti-cancer agent docetaxel. These nanoparticles were shown to specifically target the lung cancer stem-like cells, eliminate them and prevent metastasis to the liver.

Another interesting use of biopolymers is that of micelles, which serve as vehicles for delivering insoluble hydrophobic anti-cancer chemicals. Micelles are bioengineering as organized auto-assembly amphiphilic copolymers formed in a liquid, composed of solvophilic and solvophobic blocks. The core of micelles is hydrophobic, and the place where water insoluble drugs are loaded, while the outside of micelle is comprised of a hydrophilic polymer that renders the whole micelle stable and biocompatible with tissues and blood. Albumin nanocarriers were used to deliver niclosamide, a very potent anti-lung cancer agent that is normally hydrophobic, and therefore cannot be delivered systemically to the patient. In vitro trials showed that the albumin coated nanoparticles were hydrophilic and were able to deliver efficiently the agent, resulting in significant tumor inhibition and apoptosis of cancer cells. To augment the pharmacokinetics of paclitaxel, Zhang et al. generated a micelle cross-linked with amphiphilic terpolymer PEBP-b-PBYP-g-PEG formulating a shell, which was shown to increase paclitaxel intra-tracheal delivery by 2400-fold, thus preventing lung metastasis of osteosarcoma in a mouse model.

**Nanopolymers in Respiratory Gene Therapy**

Gene therapy is currently used to treat several respiratory disorders such as cystic fibrosis (CF) and acute respiratory distress syndrome (ARDS). The overall concept is to replace a mutated gene that causes the disease with a healthy copy of the gene, inhibit or knock-out a mutated gene that is malfunctioning, or introduce a new gene that helps fight the disease, providing permanent therapeutic solutions rather than treating just the symptoms. The application of biodegradable nanoparticles as gene transferring agents is being currently evaluated for a wide range of airway diseases.

CF is a lethal autosomal disease, in which the cystic fibrosis transmembrane conductance regulator gene (CFTR) is malfunctioning. The CFTR channel is present on the apical surface of epithelial cells and is critical in the chloride (Cl⁻) and bicarbonate (HCO₃⁻) transport. These channels are important for the optimal levels of water and ion components of the mucosa. CFTR gene mutations result in epithelial cell dysfunction, mucus thickening, propagation of recalcitrant bacterial populations affecting not just the lung, but also the sinuses, intestines, pancreas and other organs. In this direction glycoylated polylysine and polyethylenimine nanobeads carrying a functional CFTR gene were internalized in airway epithelial cell cultures. This was based on the fact that lectins, such as pulmonary surfactant protein A (SP-A) and D (SP-D), which are expressed in airway epithelial cells selectively bind and internalize the above glycoconjugates. In vivo studies also showed that polylysine nanobeads loaded...
with serpin-enzyme complex receptor (that binds to airway epithelia) and CFTR plasmid, restored the chloride ion transport in a CFTR knock-out mouse model. In the same way, nanoparticles conjugated with short peptides resembling integrin-binding domains successfully delivered the CFTR gene via bronchoscopic administration in a porcine CF model. Furthermore, clinical trials in CF patients have been conducted using polyethylene glycol (PEG)-substituted polylysine nanoparticles delivering intranasally the correct CFTR gene. Correction of CFTR transfer channel has been confirmed by detecting plasmid-specific DNA and mRNA while the ion transfer was corrected in seven out of twelve of the patients.

The major advantage of the biodegradable nanobeads is their small size (18-25 nm), which allows them to enter the nuclear envelope by passive diffusion and deliver the CFTR plasmid for transcription. Another advantage of their small size is the possibility to be systemically delivered via intravenous (i.v.) injection which can lead to specific lung transfection. It has been shown that DODAC:DOPE (dioleoyl-dimethyl-ammonium chloride: dioleoyl-phosphatidyl-ethanolamine) nanoparticles infused with human cytokeratin 18 gene (KRT18) gene when administered i.v. can reach the left side of the heart and travel to the bronchial circulation which supplies the alveolar capillaries of the pulmonary circulation. There, the nanoparticles deliver the KRT18 plasmid to the alveolar epithelial cells, which mitigates the CF phenotype. In addition, novel nebulization therapeutic modalities have been investigated to delivery polymeric gene vectors for several lung diseases. Alton et al showed that inhaled gene therapy has presented safety and effectiveness in phase 2b clinical trials. Liposome nanoparticles were biosynthesized containing the CFTR cDNA, nebulized and derived to the patients via inhalation resulting in significant stabilization in the lung function of CF patients. The use of biopolymers in pulmonary gene therapy is currently being evaluated and it is expected, soon, to lead to efficient therapeutic interventions that address the mechanism of airway disease, therefore providing permanent solutions.

**Biopolymers in Respiratory Distress Syndrome**

Pulmonary surfactant (PS) is a mixture consisting of 90% lipids and 10% proteins that is produced by the alveolar type II cells. It’s major bio-physiological function is to lower the surface tension that is formed at the air-liquid interface during the respiration process and prevent the alveolar collapse. Absence or deficiency of PS leads to respiratory distress syndrome (RDS). In preterm neonates, the lungs are not fully developed and the lack of PS production leads to neonatal RDS (NRDS). Natural and synthetic surfactants have been used successfully to alleviate RDS. In the case of synthetic surfactants, it has been found that supplementation with biopolymers enhances the surface activity of the synthetic lipids and prevents the inhibition of the natural PS in the lungs. For example, although dipalmitoyl-phosphatidylcholine (DPPC) and phosphatidyl-glycerol (PG) are natural components of PS, when administered exogenously in neonatal rabbit lungs, they proved ineffective. Supplementation of DPPC and PG with tyloxyol (a nonionic liquid polymer of the alkylary polyether alcohol) facilitated dispersion of the synthetic surfactant and prevention of NRDS. This synthetic surfactant supplement is FDA-approved and used in clinic (Exosurf). The biopolymers that have been tested so far with the intent to improve the surface activity of synthetic surfactants include nonionic, such as polyethylene glycol (PEG) and dextran, anionic, such as hyaluronan, and cationic polymers (e.g. chitosan). Another advantage of these polymers is that their addition reduces surfactant inhibition and improves lung function after pulmonary injury. PS inhibition takes place when surfactant encounters plasma proteins, meconium (fetal feces aspiration during gestation), and cholesterol, conditions that are associated with acute lung injury (ALI), acute respiratory distress syndrome (ARDS), NRDS, and pulmonary edema. The use of low cost, hydrophilic biopolymers as surfactant substitutes and additives has proven to be an effective approach to treat RDS.

**Nanovaccinology**

Traditional vaccines usually contain attenuated pathogens, and although they have been proven effective in preventing contagious diseases, they are not safe for immunocompromised individuals. To address these issues, components of pathogens such as bacterial lipopolysaccharides, viral proteins, or even naked DNA encoding a protective antigen, have been utilized to manufacture less reactogenic vaccines. These were proven to be less immunogenic. Although their addition resulted in enhanced immunogenicity, they also increased the topical reactions. In this direction, nanotechnology has come to introduce a new era in vaccinology. Nanovaccines are defined as the bioengineered nanoparticles that are formulated to either encapsulate within or absorb on their surface specific antigens to elicit a desired adaptive immune response. They induce cellular memory, which is central to protection
against pathogens, and generate long-term protective immunity. Nanotechnology and biomedical engineering are now facilitating cross-disciplinary research that has come to increase the biocompatibility, permeability, solubility and stability of vaccines48. Nanoparticles can be prepared by a range of biodegradable polymers such as poly-α-hydroxyacids, polyhydroxyalkanoates, poly-amino acids, or polysaccharides to generate a vesicle that either contains or displays on its surface the antigen of interest. The most commonly used biomaterials are poly-lactic-co-glycolic acid (PLGA) and poly-lactic acid (PLA)49. Also, chitosan nanoparticles apart from being biodegradable and non-toxic, they are particularly useful for vaccinology since their small size allows them to pass through the tight junctions of epithelial cells and deliver the antigen alone50. In vivo studies have shown that the delivery and uptake of nanoparticles by the antigen presenting cells such as dendritic cells (DCs) increased by 30-fold compared to the soluble antigen alone51. Another example is the chicken ovalbumin (OVA) challenge model for studying antigen-specific immune responses in mice. When mice were injected with poly-aminoacid nanoparticles encapsulated with OVA they produced significantly higher levels of IgG, IgG1, and IgG2a compared to the injections of soluble OVA. Mohr et al showed that the nanoparticles induced cellular and humoral immune responses by CD8+ and CD4+ T cell activation that produced interferon gamma (INF-γ) and polarization towards IgG2a52. Likewise, hepatitis B antigen encapsulated into a PLGA nanoparticle was shown to induce a significantly more pronounced immune response compared to the soluble virus antigen53. Moreover, shape and surface charge of nanoparticles are important for efficient delivery of antigens. Spherical nanoparticles compared to rod-like vehicles are more readily phagocytosed by macrophages and DCs. Also, positively charged biomaterials are taken up more easily by the anionic epithelial cell membranes54,55. In this concept nanoparticles composed of PLGA and polyethylene imine (PEI) were encapsulated with naked DNA encoding the Mycobacterium tuberculosis Rv1733c latency antigen. The bioengineered nanoparticles were small and positively charged and when endotracheally intubated in a mouse model, they adhered to the negatively charged lung mucosal membranes with subsequent epithelial cellular uptake. M. tuberculosis antigen was then expressed resulting in antigen presentation to DCs, T-cell proliferation, INF-γ production, secretion of interleukin 12 (IL-12), and tumor necrosis factor alpha (TNF-α) at levels comparable to lipopolysaccharides stimulation56. Taken together, the above demonstrate that biodegradable polymers are becoming the novel platforms for lung DNA vaccinations. However, given their short history in vaccinology applications, they have not established yet their safety for human use, thus further research needs to be carried out to assess their toxicity before they are incorporated in clinical trials.

IMPLANTS FOR LUNG CIRCULATION DISEASES

Without doubt, one of the most common uses of biopolymers has been the development of pulmonary cardiovascular products. In the 1990s poly (3HB) patches were developed to close pericardium during open heart surgery57 and the same material was used for augmentation of pulmonary artery58. These biodegradable patches had sufficient strength to close the arteries and drove the formation of regenerative tissue that resembled the native atrial wall. Perhaps one of the most outstanding application of biopolymers is that of the development of tissue engineered cell-seeded pulmonary valves that was successfully applied in animal models59. Researchers have used bio-absorbable poly-4-hydroxybutyric acid patches with autologous vascular cell seeding as a feasible biomaterial to augment pulmonary circulation60. Mettler et al used a mixture of polyglycolic acid and poly-4-hydroxybutyrate biopolymer and seeded the biomaterial with ovine endothelial progenitor and mesenchymal stem cells for 5 days. The patches when implanted into the ovine pulmonary artery showed the successful creation of artificial bioengineered blood vessel61.

DISCUSSION

Biopolymers are the natural metabolite products formed during the life cycle of animals, bacteria, fungi and plants. Because of their high biocompatibility, and their non-toxic degradation products they have come to be ideal biomaterials that found applications in a number of airway diseases, as they are summarized on Figure 2. We are expecting that in the near future a number of biomaterials will be utilized to bioengineer fully functional lung tissues from the very own stem cell lines of the recipient. It is without doubt that in the approximate future biopolymers will continue to find more biomedical applications in airway disease.
COMPETING INTERESTS

The authors declare that they have no competing interests.

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DECLARATIONS

Ethics approval

This review article was evaluated and approved by the Arizona State University and Aristotle University.

Consent for publication

Not applicable.

Authors’ contributions

GTN reviewed the relevant literature, designed the structure of the review article, integrated and synthesized published data, contributed to manuscript writing, prepared figures. AAP, contributed to manuscript writing, prepared figures, contributed to manuscript writing, and provided oversight to the entire review progress.

FIGURE 2. Schematic representation of biomedical applications of biopolymers in airway disease.
ΠΕΡΙΛΗΨΗ

Βιοϊατρικές εφαρμογές των βιοπολυμερών στη νόσο των αεραγωγών

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Ο όρος “νόσος των αεραγωγών” περιγράφει διάφορα γεγονότα που οδηγούν σε καταστροφή του πνευμονικού ιστού, κακή αιματική κυκλοφορία, και απόφραξη των αεραγωγών που εμποδίζουν τη λειτουργία των πνευμόνων. Πολυμερικά βιοϋλικά που είναι βιοαποικοδομήσιμα έχουν αναδυθεί ως σημαντικά επιτεύγματα της σύγχρονης ιατρικής. Σε αυτήν την ανασκόπηση, επιδιώξαμε να διερευνήσουμε το κλινικό δυναμικό των βιοπολυμερών στην ασθένεια των αεραγωγών. Αρχικά συζητούμε συνοπτικά τη βιοσύνθεση των βιοϋλικών και τη χρήση τους σε ικριώματα των ιστών των πνευμόνων, στη μηχανική των μοσχευμάτων χονδρών και τη χρήση τους ως υποστρώματα για in vitro καλλιέργεια αναπνευστικών επιθηλιακών κυττάρων.

Στη συνέχεια συζητάμε τη χρήση τους ως βιοαπορροφήσιμα νανοδομημένα συστήματα χορήγησης φαρμάκων που καταπολεμούν τον καρκίνο του πνεύμονα καθώς και την πρόληψη της μετάστασης με στόχο την παρεμπόδιση των καρκινικών πνευμονικών κύτταρων. Επιπλέον, αναφέρουμε στη χρήση των βιοπολυμερών μαζί με λιπίδια ως υποκατάστατα του πνευμονικού επιφανειοδραστικού παράγοντα στο σύνδρομο οξείας αναπνευστικής δυσχέρειας. Προτείνουμε τη χρήση βιοπολυμερών ως χειρουργικά εμφυτεύματα στη λειτουργία των πνεύμονων. Λέξεις - Κλειδιά: πολυhydroxyalkanoates, νόσος των αεραγωγών, καταστροφή πνευμονικού ιστού, συστήματα απελευθέρωσης φαρμάκων, νανοεμβολιολογία

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Physiotherapy in cystic fibrosis
A comprehensive clinical overview

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Key words:
- Physiotherapy
- Cystic Fibrosis
- Airway Clearance
- Chest Physiotherapy
- Exercise

SUMMARY
Physiotherapy remains the cornerstone of cystic fibrosis (CF) management alongside medical treatment. Traditionally, physiotherapy intervention focussed on airway clearance during the clinically stable stage and chest infections. Research evidence consistently supports greater mucus clearance with chest physiotherapy compared to cough alone or no treatment. Various methods and techniques of airway clearance have been developed and investigated, and data suggest that most of them are of similar effectiveness. Nowadays physiotherapy management also extends to other areas, supported by studies and clinical practice. The physiotherapists plan, supervise and follow-up systematic exercise or personalised rehabilitation programs, which, similarly to airway clearance, are recommended in all patients with CF. Furthermore, based on a comprehensive assessment, physiotherapists incorporate the management of accompanying musculoskeletal problems such as back pain and postural disorders, as well as urine incontinence issues. In the era that aims to improve quality of life, it is essential that physiotherapists are aware of specific conditions that might affect the management of CF. Their role is to work alongside and within the CF multi-disciplinary team throughout patient’s treatment and consistently support the patient and carers, in particular whilst on clinical pathways of the lung transplantation and palliative care.


INTRODUCTION
Cystic fibrosis (CF) is a recessive genetic disease that affects the patient on multiple systems, with profound manifestations in the respiratory and digestive systems. It is characterised by the mutation and therefore dysfunction of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR). This protein mainly functions as an ion channel, regulating fluid volume on epithelial surfaces via chlorine secretion and inhibition of sodium resorption. In the airways of the patients with CF, dysfunction
of the CFTR results in periciliary liquid layer depletion\(^2\). Clinically, patients with CF present abnormal consistency and high volumes of sputum, cough, dyspnoea, bronchiectasis and weight loss. As the survival of these patients is increasing, it is crucial that health care professionals address symptoms and support individuals in evolving issues developed throughout their life span.

Physiotherapy is an integral part of the therapeutic management of CF patients, both at the clinically stable stage of the disease and during respiratory infections. In the past, physiotherapy was focused on airway clearance, also known as chest physiotherapy, by teaching or applying methods such as the postural drainage with or without the additional application of manual techniques\(^3\). Postural drainage of the tracheobronchial tree uses specific gravitational positions to assist mucus mobilisation downwards (towards the mouth) within the airways. Manual techniques (percussions, vibrations and/or shakes) use mechanical forces to assist the detachment of mucus from the airway epithelium and its mobilisation. Nowadays, the choice of airway clearance techniques has been expanded to methods such as the autogenic drainage, the active cycle of breathing techniques (ACBT), the use of positive expiratory pressure (PEP) devices with or without oscillation, and others. Still, modern physiotherapy in CF also includes the assessment of the cardiovascular system and improvement of the patient’s fitness level, muscle strength and endurance through exercise, as well as specialised interventions to improve musculoskeletal symptoms of pain, posture and incontinence\(^4\).

**Physiotherapy**

**Airway clearance**

Patient education, application and monitoring of the airway clearance techniques remain the main physiotherapy treatment for patients with CF\(^5\). Physiotherapists facilitate the establishment of an individualised airway clearance routine by supporting patients and their families to establish regular regimes during a clinically stable stage and have an escalation plan for disease exacerbations\(^5\). Airway clearance is usually performed on a daily basis and as required. *The selected method applied, duration and frequency of each session are tailored to the patient, their general health condition and the severity of the disease.* For instance, airway clearance becomes more regular during exacerbations or hospitalisations\(^6\). Hospitalisations also provide an opportunity for physiotherapists to re-assess the effectiveness of daily airway clearance and provide appropriate feedback and guidance for improving the patient’s usual technique prior to discharge.

Table 1 presents the main categories of airway clearance techniques and methods in CF. These can be used in isolation or in combination regimes. Assessment of effectiveness is based on measuring sputum volume or weight, lung function by spirometry, frequency of hospitalisations and quality of life. Airway clearance is extensively supported in the literature when compared to no airway clearance or cough alone\(^4,7\). A recent systematic review supported a significant increase in the amount of sputum (wet or dry) in the patient groups that applied airway clearance using postural drainage with or without the addition of manual techniques or using PEP, compared to spontaneous cough or not using any technique\(^7\). The weight of the sputum was higher after the application of the active cycle of breathing techniques compared to the use of the flutter (an oscillating PEP device) or high frequency chest wall oscillation (vest)\(^8\). The weight of the sputum expectorated was greater after using the PEP mask compared to autogenic drainage, postural drainage positions and their combination, although this difference was short-term (up to one week)\(^9\). On the other hand, there was no difference in the amount of the expectorated mucus after autogenic drainage compared to the flutter, or between the high frequency chest wall oscillation compared to the autogenic drainage or the PEP mask for longer time-intervals\(^10,12\).

Systematic reviews did not show significant differences in the lung function (FEV\(_1\)) of adult patients fol-

**Table 1.** Common airway clearance techniques and methods.

<table>
<thead>
<tr>
<th>Airway clearance techniques</th>
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<tr>
<td>• Postural drainage</td>
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<td>• Manual techniques</td>
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<td>• Active circle of breathing techniques (ACBT)</td>
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<tr>
<td>• Autogenous drainage (AD)</td>
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<tr>
<td>• Positive expiratory pressure (PEP) devices (PEP mask, Pari-PEP, etc)</td>
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<tr>
<td>• Positive expiratory pressure (PEP) devices with oscillation (flutter, acapella, cornet, etc.)</td>
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<tr>
<td>• Intermittent Positive Pressure Breathing (IPPB)</td>
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<tr>
<td>• High frequency chest wall oscillation (HFCWO) or vest</td>
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<tr>
<td>• Non-invasive mechanical ventilation (NIV)</td>
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<td>• Aerobic exercise</td>
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lowing the use of PEP, when assessed patients prior and immediately after a physiotherapy session or up to 3 months later7,10,11,12. Additionally, the lung function did not change after applying the active cycle of breathing techniques in combination with the PEP mask, postural drainage with or without manual techniques, or the high frequency chest wall oscillation12. However, treatment in children and adolescents that was applied up to one year showed 6% increase in FEV1 with the use of PEP13.

Regarding the hospitalisation frequency, no differences were found for those who practiced the active cycle of breathing techniques compared to the postural drainage with or without manual techniques12. The number of hospitalisations, however, was lower for those who used PEP than the patients who used the flutter (5 vs 18 hospitalisations, respectively)10. Similarly, fewer patients used intravenous antibiotics from the group that used PEP devices, compared to the group of the high frequency chest wall oscillation13.

For the quality of life, there is no difference amongst techniques and devices, such as the postural drainage with or without manual techniques, active cycle of breathing techniques, autogenic drainage, PEP mask, flutter, and cornet10,12,13. However, patients preferred the PEP mask for long-term use (>1 month), and also preferred seating instead of using postural drainage positions10,11,13. Autogenic drainage was preferred among children between 12-18 years old, compared to postural drainage in combination with manual techniques14.

Important factors for the success of the selected airway clearance plan are the compliance to treatment and patient satisfaction. Factors that increase the rate of compliance are good patient knowledge of the technique and confidence in its application, independence and preference15,16. Evidence indicate that patients who receive help, those who produce more sputum, and children with CF whose parents believe in the necessity of treatment are those with higher compliance in airway clearance17,18.

Airway clearance adaptations

Mucolitics and other agents

Patients with CF often receive medications that aim to increase the effectiveness of airway clearance, such as nebulised hypertonic saline (3% to 7% NaCl), dornase alpha (DNase), and mannitol. The use of inhaled hypertonic saline (osmotic pressure >0.9% NaCl) in patients with CF is considered to improve the rheological characteristics of sputum and increase the hydration of the airway epithe-

lium; thus, increase the sputum motility and facilitate the mucus clearance19. There is good evidence that the use of hypertonic saline reduces the incidence of respiratory infections, increases FEV1, and improves the quality of life, although the changes are not maintained in the long term (48 weeks)20,21. During the hospitalisation of patients with CF, hypertonic saline improves the chances of quick return of the lung function (FEV1) to pre-infectious levels22. With regards to timing the hypertonic saline administration, a recent systematic review supports its use before or during the performance of airway clearance, rather than its administration afterwards23. If the prescribed doses are two, it is recommended to administer one in the morning and one in the evening, and if the patient receives a single dose this is given at a convenient time chosen by the patient23.

Dornase alpha (DNase) is a recombinant human deoxyribonuclease that reduces sputum viscosity by selectively hydrolysing the large extracellular DNA molecules contained in the mucus into smaller structures, thereby increasing the potential for its elimination24. This drug is administered via a jet-nebuliser device and has been shown to reduce the incidence of respiratory infections, increase respiratory function, and improve quality of life24. With regards to timing its administration, it appears that using DNase before or after airway clearance does not have any difference in improving lung function (FEV1 and FVC) or patient’s quality of life25,26. In clinical practice, physiotherapy often follows the proposed guidelines of the pharmaceutical company to perform airway clearance 30 minutes after the DNase administration27.

Inhaled mannitol is a naturally occurring sugar alcohol which enhances osmosis, causing mucous hydration28. Inhaled mannitol is administered as dry powder (capsules) using an inhaler. As demonstrated by two 26-week multi-centre studies with a total number of 600 participants with CF, inhaled mannitol improves the respiratory function of patients but does not improve their quality of life29,30. Although its use usually precedes airway clearance in clinical practice, there is no research data to compare different timings of administration.

Haemoptysis

Haemoptysis is a major change in the patient’s clinical presentation and may be life-threatening. The physiotherapy assessment should include questions about sputum description and reference to current or past haemoptysis episodes. Active frank haemoptysis (>100-1000 ml haemoptysis in 24 hours or 48 hours) is treated
exclusively medically, e.g. with bronchial embolisation of the arteries or thoracic surgery, while the airway clearance treatment is temporarily discontinued\textsuperscript{31,32}. In moderate or low haemoptysis, physiotherapists, in collaboration with the medical team, decide whether or not it is appropriate to continue airway clearance using clinically reasoning. If the treatment is appropriate and safe to continue, then the active cycle of breathing techniques or autogenic drainage is often selected over other techniques.

Pneumothorax

Spontaneous pneumothorax is a common complication in patients with CF. It is associated with a reduction in pulmonary function and 50-90% chance of recurrence\textsuperscript{32,33}. If the pneumothorax occurs for the first time and it is small, then it can be treated conservatively with oxygen supply\textsuperscript{34}. In patients continuing airway clearance, it is suggested to liaise with the medical team for adding humidification to the oxygen supply and ensuring adequate analgesia for the duration of the treatment sessions\textsuperscript{35}. In the case of large pneumothorax (>2 cm between parietal pleura and visceral pleura) or recurrent pneumothorax, chest drainage is performed using thoracic catheters, while patients might get pleurodesis in resistant cases\textsuperscript{34}. Positive pressure devices such as PEP, flutter and acapella are contraindicated in the presence of pneumothorax\textsuperscript{34}. Regarding physical activity, patients need to be engaged with moderate activities but should avoid bearing weights over 2 kg or strenuous aerobic exercise for a period of two to six weeks after the complete drainage of the pneumothorax\textsuperscript{34}.

Exercise

Exercise is an integral part of the comprehensive physiotherapy intervention for patients with CF\textsuperscript{36}. American College of Sports Medicine guidelines advocate 3-5 sessions of moderate exercise per week, with the aim to adopt exercise as a way of living\textsuperscript{37}. Benefits of specific exercise modalities in cystic fibrosis are yet to be identified in methodologically strong studies\textsuperscript{38}. Despite research interest, evidence has not established the effectiveness of inspiratory muscle training on this group of patients, therefore this is currently not routinely incorporated in the CF treatment. In the clinical setting, the assessment of patients with CF uses simple and cost-effective exercise field tests, such as the 6-minute walk test (6MWT) and the incremental shuttle walk test (ISWT), whilst the level of dyspnoea is assessed using the Borg dyspnoea scale\textsuperscript{39}.

Exercise can theoretically assist airway clearance through the kinetic forces and vibrations generated within the airways, but it cannot substitute for the formal airway clearance\textsuperscript{40}. When compared to airway clearance techniques, moderate aerobic exercise leads to less mucus expectoration\textsuperscript{41}. Also, exercise as a single agent does not increase cough immediately after its completion, although it improves the subjective ease of sputum clearance\textsuperscript{42}. Clinically, exercise is mainly used additionally to airway clearance, as a means to improve the exercise capacity of the patient and is usually performed before the implementation of airway clearance.

Exercise considerations

Musculoskeletal and postural issues

Back and thoracic pain are frequently reported in patients with CF, although they do not have an effect on lung function\textsuperscript{43,44}. Higher thoracic kyphosis is associated with lower lung function, but nowadays it is more uncommon compared to a few years ago\textsuperscript{45}. Low bone density and osteopenia is also a common issue in patients with CF\textsuperscript{46,47}. Counselling and appropriate exercise programs from physiotherapists can potentially address and improve these postural and structural issues\textsuperscript{36}.

Urinary incontinence

Surveys show that urinary incontinence in patients with CF is reported in 30% to 68% of women or girls and 5% to 16% of men or boys\textsuperscript{48-51}. The dynamic pressure created during coughing is potentially a key mechanism of CF urinary incontinence, although it may not be the only one\textsuperscript{52}. Coughing, sneezing, laughing and spirometry are among the activities that trigger urinary incontinence incidents\textsuperscript{53}. Incontinence worsens during respiratory infections and has been associated with poorer quality of life and higher anxiety and depression scores\textsuperscript{51,54,55}. Assessing incontinence using screening tools and clarifying questions should be an integral part of the CF physiotherapy assessment, regardless of gender\textsuperscript{56}. Physiotherapy treatment of urinary incontinence includes counselling and specialised training involving pelvic floor exercises, such as Kegel exercises\textsuperscript{55,57,58}.

Diabetes mellitus

Diabetes mellitus is associated with CF and is the most common comorbidity of the disease, occurring in up to 20-50% of adult patients\textsuperscript{59-61}. This comorbidity requires the co-operation of the physiotherapists with the
endocrine team, especially for the patients who require insulin therapy\(^6\). Additionally, the presence of diabetes mellitus needs to be considered in the physiotherapy plan, mainly in the exercise prescription and performance. In this case, the proper scheduling of the meal times or insulin intake is essential.

**Quality of life**

Over time and as the CF severity and symptoms progress, the quality of life of patients is deteriorating. Females with CF often report poorer quality of life compared to their male age-matched peers\(^6\). Although the correlation between lung function and quality of life is weak to moderate, patients with better lung function report higher quality of life\(^5\). Also, the presence of *Pseudomonas aeruginosa* and frequent respiratory infections appear to have a negative impact on the quality of life of patients\(^5\).

Researchers and clinicians can use a number of validated questionnaires for the assessment of quality of life in people with CF. Those include: generic questionnaires or questionnaires for a specific disease symptom, such as the Short Form-36 (SF-36) and the Leicester Cough Questionnaire, respectively\(^6,6\); disease-specific questionnaires, such as the Manchester Questionnaire, the Cystic Fibrosis Questionnaire-Revised and the Cystic Fibrosis-Quality of Life\(^6,6\); and questionnaires for babies and children of young age, such as the Modified Parent Cystic Fibrosis Questionnaire-Revised\(^6\).

**Special considerations**

**Long term oxygen therapy and non-invasive ventilation**

A recent systematic review in patients with CF did not show long-term benefits from the long-term oxygen therapy, in survival, respiratory function or cardiovascular health, although it showed improved school or work attendance rates\(^7\). When oxygen is administered during exercise only, it helps to improve oxygenation, reduces the feeling of dyspnoea and increases the duration of the exercise\(^7\). However, supplemental oxygen during exercise in patients with initially low arterial oxygen values appears to cause hypercapnia in the short term (PCO\(_2\) up to 16 mmHg)\(^7\). Also, oxygen therapy during sleep improves oxygenation, but is accompanied by small hypercapnia\(^7\). The use of supplemental oxygen should follow the established clinical guidelines that are based on hypoxia (PaO\(_2\) ≤55 mmHg or 60 mmHg) and the presence of clinical symptoms\(^7\).

Non-invasive ventilation (NIV) is used in patients with CF on respiratory failure, hypoventilation during sleep, as well as a bridge to lung transplantation\(^6\). For patients with severe clinical presentation where airway clearance causes fatigue and high levels of dyspnoea, NIV can be used to assist airway clearance\(^7\). The use of NIV during the physiotherapy session facilitates mucus expectoration and reduces the sensation of dyspnoea during the treatment compared to other techniques particularly for patients with low lung function\(^7\). However, the long-term effects of NIV on airway clearance need further investigation\(^6\).

**Paediatric population**

Choosing a treatment plan for children with CF is based on age, clinical presentation and certain social criteria\(^7\). There is no agreement on the most appropriate starting age for airway clearance. A proposal for early disease management (pre-symptomatic) is to carefully monitor the clinical presentation of children and adopt an active treatment plan following the onset of symptoms\(^7\). At young ages, where the child can not follow instructions and cooperate, assisted autogenic drainage or PEP devices with a child mask can be used. Physiotherapists are also responsible for educating the child’s parents or carers for appropriate evaluation of the child’s symptoms and treatment implementation as required\(^7\). Postural drainage with tilt (head-down positions) is no longer advised for babies, as it has been shown to increase the gastroesophageal reflux\(^8\).

As children grow older, they can more actively participate in their treatment. Children over 3 years old can also use an airway clearance game, the bubble PEP. This is a positive-pressure home-made device, where children are encouraged to generate soap bubbles by breathing out through a small plastic tube and into a bottle of soapy water\(^8\). According to the UK Cystic Fibrosis Foundation, at the age of 6 years or more, the use of nebulised hypertonic saline can be initiated in combination with airway clearance\(^8\). Also, at all ages, activity games and engagement with exercise are encouraged and used, for instance racing, trampolines and exercises using a gym ball\(^8\).

**Palliative care**

CF is a disease that limits life expectancy and requires discipline and consistency to many hours of daily treatment. As a result, its psychological impact should not be ignored\(^8\). If patients are in respiratory failure and in lung transplantation list, pulmonary rehabilitation is the treatment priority, alongside the aim to relieve symptoms. Working in line with the patient’s wishes is very impor-
tant, particularly during the palliative care stage. Airway clearance of less active patient participation (eg. postural drainage), massage and some dyspnoea relieving positions could be applied during this stage, if they provide comfort to the patient.

CONCLUSIONS

CF management is highly demanding, mainly aiming to the reduction and treatment of chest infections, improvement of quality of life and increase of life expectancy. Physiotherapy is an integral part of the patient’s daily treatment routine, and additionally to airway clearance other important issues should be addressed. International clinical guidelines suggest access to specialised physiotherapy care both during a clinically stable stage of the disease and during respiratory infections. At the clinically stable stage, patients should be evaluated by physiotherapists every 3-6 months to re-evaluate and optimize their treatment plan. During respiratory infections, physiotherapy interventions are intensified according to the clinical presentation. Although in CF airway clearance is the cornerstone of physiotherapy treatment, physiotherapists work beyond the respiratory system and play an important role in the management of other issues, mainly using individualised exercise programmes. The exercise programmes need to be tailored to patient-related needs and issues, such as pain, diabetes and incontinence. This way, the patient-centred and individualised treatment follows the international standards and clinical guidelines.

CONFLICT OF INTEREST DECLARATION

No conflict of interest.

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ΠΕΡΙΛΗΨΗ

Φυσικοθεραπεία στην κυστική ίνωση: Μια περιεκτική κλινική ανασκόπηση

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Η φυσικοθεραπεία παραμένει μια από τις κύριες μεθόδους διαχείρισης της κυστικής ίνωσης, σε συνδυασμό με την ιατρική θεραπεία. Παραδοσιακά, η φυσικοθεραπεία επικεντρώνονταν στον τραχειοβρογχικό καθαρισμό κατά τη διάρκεια της κλινικά σταθερής φάσης και των αναπνευστικών λοιμώξεων, με τα ερευνητικά δεδομένα να υποστηρίζουν την αποτελεσματικότητά της συγκριτικά με τον βήχα ή τη μη θεραπεία. Διάφορες μέθοδοι και τεχνικές τραχειοβρογχικού καθαρισμού έχουν αναπτυχθεί και διερευνηθεί, και τα δεδομένα προτείνουν ότι οι περισσότερες από αυτές είναι παρόμοιες αποτελεσματικότητας. Επιπλέον, σήμερα, οι έρευνες και η κλινική πρακτική επεκτείνουν τη φυσικοθεραπευτική διαχείριση πέραν του αμιγώς αναπνευστικού συστήματος. Οι φυσικοθεραπευτές σχεδιάζουν, επιβλέπουν και επανελέγχουν τη συστηματική άσκηση ή εξατομικευμένο πρόγραμμα αποκατάστασης, που ομοίως με τον τραχειοβρογχικό καθαρισμό συστήνεται σε όλους τους ασθενείς με κυστική ίνωση. Ακόμα, όταν χρειάζεται και με βάση μια ολοκληρωμένη αξιολόγηση, η φυσικοθεραπεία πραγματεύεται στη διαχείριση συνοδότων μυοσκελετικών προβλημάτων όπως σφυγμικής, εργονομικών προβλημάτων στάσης και ακράτειας. Σε μια εποχή που στοχεύει στη βελτίωση της ποιότητας ζωής, οι φυσικοθεραπευτές είναι απαραίτητο να γνωρίζουν τις ειδικές περιπτώσεις που επηρέαζουν τη διαχείριση της κυστικής ίνωσης. Ο ρόλος τους είναι να εργάζονται σε συνεργασία με την πολυ-επιστημονική ομάδα για την υποστήριξη των ασθενών και του περιβάλλοντός τους, ιδιαίτερα όταν οι ασθενείς είναι σε αναμονή για μεταμόσχευση ή κατά την παρηγορητική φροντίδα.


Λέξεις - Κλειδιά: φυσικοθεραπεία, κυστική ίνωση, τραχειοβρογχικός καθαρισμός, αναπνευστική φυσικοθεραπεία, άσκηση
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Reversed halo sign in community acquired pneumonia
A case report

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- Reversed halo sign
- Atoll sign
- HRCT
- Community acquired pneumonia

SUMMARY
Reversed halo sign (RHS) is defined as central annular ground-glass opacity surrounded by a ring of denser consolidation at least 2mm thickness. It was first described by Voludaki et al. in a case report of two COP cases and later concluded that it was specific to cryptogenic organizing pneumonia. Since then, the RHS was associated with a wide range of pulmonary diseases: pulmonary fungal infections, tuberculosis, community – acquired pneumonia, sarcoidosis, pulmonary neoplasms, Wegener granulomatosis, pulmonary infraction and other diseases. We report a patient case of community-acquired pneumonia with of RHS on HRCT, and we review the literature on this radiological sign. We present a 70 years-old male, who was admitted to emergency department with lower tract respiratory infection symptoms, HRCT was performed and revealed multiple round ground-glass opacities fringed with peripheral consolidation in both lungs. The patient was diagnosed with community-acquired pneumonia and treated successfully with respiratory quinolone. Unfortunately, the infectious agent was not determined, as well as bronchoscopy with BAL was not helpful for diagnosis. At follow up, in 21 days and 4 months, the patient remained asymptomatic, and chest CT revealed a clear improvement. Finally, reversed halo sign has been reported in a wide range of conditions, and investigation of its aetiological factors is required.


INTRODUCTION
Reversed halo sign, also known as atoll sign, is defined as central ground-glass opacity surrounded by denser consolidation of crescentic or ring shape of at least 2mm thickness. It was first described on high-resolution CT (HRCT) as being specific for cryptogenic organizing pneumonia (COP).
Since then, the reversed halo sign was associated with a wide range of pulmonary diseases: pulmonary fungal infections, pneumocystis pneumonia, tuberculosis, community-acquired pneumonia, lymphomatoid granulomatosis, Wegener granulomatosis, lipoid pneumonia, sarcoidosis, pulmonary neoplasms, pulmonary infraction and following radiation and radiofrequency therapy of pulmonary malignancies.

**CASE PRESENTATION**

We are reporting a 70 years-old male patient who was admitted to our hospital with a one-week history of fever and non-productive cough. The patient also complained for anorexia and weight loss. He was treated with antibiotics (Amoxicillin/Clavulanic Acid Tb 875/125mg 1x2 + Clarithromycin Tb 500mg 1x2) five days before his admission by his general practitioner. Due to the persistence of fever he was referred to our clinic. His past medical history was unremarkable for any chronic medical illness. He was a smoker of 50 pack/years and denied ethanol, drug abuse and recent travel. No drug allergies were noted.

At presentation the patient’s body temperature was 37.8°C, blood pressure was 120/75 mm Hg and percutaneous oxygen saturation was 97% in room air. His heart rate was 100 to 110 beats per minute with a sinus rhythm revealed on ECG. Respiratory rate was 18 to 20 breaths per minute. His heart sounds were normal with no murmurs or extra sounds. Auscultation revealed coarse crackles over the posterior right lung. There was no clubbing, cervical or axillary lymphadenopathy, skin lesions or joint swelling. Physical examinations of the rest systems did not provide any significant information.

The patient’s white blood count was 5,760 cells/mm3 (lymphocytes = 20%, neutrophils = 69%, and atypical = 8%). CRP level was 8.4 mg/L.

At presentation the chest X-ray showed a consolidative pattern in the right middle and lower lung fields. Chest computed tomography (CT) revealed multiple round ground-glass opacities fringed with consolidation in both lungs, namely the “reversed halo sign” (Figure 1).

Afterwards bronchoscopy with BAL was done and didn’t reveal any remarkable endoscopic findings (bronchoalveolar lavage fluid (BALF) cell analysis: alveolar macrophages 84%, CD4+ 5%, CD8+ 3%, CD4+/CD8+ =1.6, neutrophils 5%, squamous epithelial cells 3%). Tests for antinuclear antibody (ANA) and anti neutrophil cytoplasmic antibody (ANCA) were negative. The Legionella and Pneumonococcal antigen urine testing were negative. Blood cultures were negative. Sputum cultures were negative.

![FIGURE 1. Multiple round ground-glass opacities fringed with consolidation in both lungs.](image1)

![FIGURE 2. After 21 days.](image2)

![FIGURE 3. After 4 month.](image3)
not performed, since the patient had no expectoration. The patient was diagnosed with community- acquired pneumonia and treated successfully with Moxifloxacin i.v. (400mg/d), without receiving corticosteroids. The symptoms were improved dramatically and he became febrile within the fourth day. At follow up the patient remained afebrile. Computed tomography of the chest (after 21 days and 4 months) revealed a clear improvement (Figure 2, Figure 3).

REVIEW

The reversed halo sign was first described and associated with COP, but it is not specific to this disease. A wide spectrum of conditions can manifest with the reversed halo sign on chest HRCT.

The reversed halo sign (RHS) is characterized by a focal area of ground-glass opacity surrounded by a more or less complete ring of consolidation on high-resolution CT (HRCT).

Sometimes RHS can have specific morphological findings helpful in differential diagnosis as RHS with thickened rim and reticulation or “bird nest sign” and RHS with micronodules. The reticular RHS is linked with invasive fungal disease. RHS with micronodules is related to active granulomatous disease, mainly tuberculosis, but also paracoccidioidomycosis (PCM) or cryptococcosis or “bird nest sign” and RHS with micronodules. The reticular RHS is linked with invasive fungal disease. RHS with micronodules is related to active granulomatous disease, mainly tuberculosis, but also paracoccidioidomycosis (PCM) or cryptococcosis and non-infectious granulomatous diseases such as sarcoidosis.

The presence of RHS on HRCT can be useful to narrow the differential diagnosis. Analyzing the patient’s clinical history and additional CT findings is helpful for the final decision and treatment.

There is a spectrum of infectious, neoplastic, non-infectious/non-neoplastic diseases that may appear as RHS on HRCT. Various clinical situations that can guide the clinicians were described.

Clinical signs and symptoms of pulmonary infection

In immunosuppressed patients opportunistic fungal diseases should be included in the differential diagnosis. Opportunistic invasive fungal pneumonias (IFPs) have high morbidity and mortality. The most common IFP is an invasive pulmonary aspergillosis (IPA). Other angioinvasive moulds, such as Zygomycetes species are encountered in immunosuppressed patients. In cases of IFP, the RHS is an early sign that results from pulmonary infarct. Other findings include nodules and pleural effusion.

The RHS has been described in up to 10% of patients with PCM. PCM is frequent mycosis in Latin America. The HRCT findings of patients with pulmoparacoccidiiodomycosis include ground-glass areas, small centrilobular nodules, cavitated nodules, and areas of emphysema.

In patients from areas with high rates of Mycobacterium tuberculosis infection (TB), pulmonary tuberculosis should always be included in the differential diagnosis. Additional CT findings can help the radiologist: centrilobular nodules and tree-in-bud opacities, as well as subcarinal and hilar lymphadenopathy, areas of consolidation with cavitation. It is remarkable that areas of consolidation have usually upper lobe distribution in these cases.

Pneumocystic Jiroveci Pneumonia (PJP) is the most common opportunistic infection in HIV-positive patients. The RHS has been described in AIDS patients with pneumocystis pneumonia.

The RHS has been reported in cases of bacterial, pneumococcal, psittacosis or legionella pneumonias. Since infection can cause organizing pneumonia, it is possible that in some of the reported cases of bacterial pneumonia the RHS was a part of secondary organizing pneumonia, provoked by the inflammatory damage.

Known primary neoplasm

The RHS has been described as an early secondary finding of radiofrequency ablation (RFA) of pulmonary nodules. The central GGO area is corresponded to an area of coagulative necrosis of the nodule, whereas peripheral consolidation is corresponded to fibrotic tissue.

Radiation-induced lung disease (RILD) is common following radiation therapy of the thorax. The RHS may be seen during the acute phase of RILD, in the first 4-12 weeks after treatment. It is probably related to inflammatory process or pulmonary necrosis related to radiation, or secondary organizing pneumonia triggered by radiation injury of the lung.

In patients under chemotherapy, multiple RHS lesions may correlate with non-specific interstitial pneumonia (NSIP) or organizing pneumonia linked with drug-induced toxicity.

In patients with a known primary malignancy RHS lesions may appear as atypical presentation of metastatic disease. The presence of new RHS lesions in these patients should be examined for lung metastatic progression.

Patients with vascular or thromboembolic disease

Patients with pulmonary embolism (PE) may pres-
ent RHS on CT in case of pulmonary infarction. The RHS in patients with pulmonary infarction translates central coagulative necrosis with peripheral rim of collagen tissue produced by fibroblasts\textsuperscript{16,17}.

The RHS has been described in patient with Wegener’s granulomatosis, in association with lung nodules, ground-glass opacities and cavitary lesions. RHS in this condition represent an intermediate stage that preceded cavitation\textsuperscript{18}.

Asymptomatic patient or with subacute clinical symptoms

RHS has been described as an atypical manifestation in sarcoidosis. Sarcoidosis is a granulomatous disease. In 90% of cases lungs and intrathoracic lymph nodes are affected. RHS in sarcoidosis can represent either non-caseating granulomatous inflammation or secondary organizing pneumonia\textsuperscript{7}.

Cryptogenic organizing pneumonia (COP) is the most common lung disease described in immunocompetent patients with the RHS\textsuperscript{1}. This sign can also be seen in cases of secondary organizing pneumonia. Histopathologically, the central ground-glass opacity of the RHS corresponds to alveolar septal inflammation; the peripheral consolidation represents organizing pneumonia within the alveolar ducts\textsuperscript{6}.

Non-specific interstitial pneumonia (NSIP) is an interstitial lung disease that may be idiopathic, but is more commonly associated with collagen vascular disease, hypersensitivity pneumonitis or drug toxicity\textsuperscript{7}. The RHS correlates with interstitial inflammation that predominates in the middle and lower lung. HRCT in patient with NSIP reveal also reticular pattern, areas of consolidation and traction bronchiectasis.

The RHS has been described by Kanaji et al. in case of exogenous lipoid pneumonia after inhaling spray paint. In this case RHS represented organizing pneumonia resulting from lipid pneumonia\textsuperscript{19}.

Pulmonary adenocarcinoma may present as an area of consolidation, a single node or as multiple nodules. The RHS is an uncommon presentation of lung adenocarcinoma\textsuperscript{7}.

Lymphomatoid granulomatosis (LG) is associated with Epstein-Barr virus (EBV) which mainly affects the lungs. In this case, the RHS corresponds to area of aerated parenchyma with a peripheral ring of lymphomatoid vascular invasion\textsuperscript{2}.

CONCLUSION

A wide variety of diseases, infectious and noninfectious, may present with the reversed halo sign on chest CT. The two most commonly associated diseases are the organizing pneumonia and invasive fungal pneumonia. The patient’s history and clinical data in combination with the additional radiological findings should help to narrow the differential diagnosis. Although a biopsy is needed in many diseases with RHS on HRCT, it can be avoided in certain scenarios.

In the clinical case reported above, the RHS on chest CT was related with inflammatory process, provoked by infection. The diagnosis of CAP was based on clinical presentation, laboratory tests (acute phase protein), and clinical improvement after treatment with antibiotics only. Unfortunately, the infectious agent was not determined, as well as bronchoscopy with BAL was not helpful, since it was performed in the convalescent phase. Nevertheless, we assume that the causative agent provoked organizing pneumonia and persistent inflammation in lung parenchyma, causing prolonged symptoms.
πνευμονικά νοσήματα: μυκητιασικές πνευμονικές λοιμώξεις, φυματίωση, πνευμονία της κοινότητας, σαρκοειδώση, κοκκιωμάτωση Vegener, πνευμονικά έμφρακτα, νεοπλάσματα πνεύμονα κ.α. Στο παρόν άρθρο παρουσιάζεται περίπτωση ασθενούς με πνευμονία της κοινότητας με ακτινολογική εικόνα ανάστροφου σημείου της άλω στην αξονική τομογραφία υψηλής ευκρίνειας και γίνεται ανασκόπηση της βιβλιογραφίας. Πρόκειται για ασθενή ηλικίας 70 ετών που προσήλθε στο ΤΕΠ με συμπτώματα λοίμωξης κατώτερου αναπνευστικού, υπεβλήθη σε HRCT, η οποία ανέδειξε πολλαπλές δακτυλιοειδείς σκιάσεις τύπου θολής υάλου με πυκνωτική περιφερική ζώνη. Αντιμετωπίστηκε επιτυχώς με αναπνευστική κινολόνη, ως περιστατικό πνευμονίας της κοινότητας με μη ταυτοποιημένο αιτιολογικό παθογόνο, δεδομένου ότι ο εργαστηριακός έλεγχος δεν απομόνωσε υπεύθυνο μικροοργανισμό, όπως επίσης αργότερα η βροχοσκόπηση με βροχοκυψελικό έκπλυμα δεν ανέδειξε ιδιαιτέρα παθολογικά ευρήματα. Κατά την επανεκτίμηση σε 21 ημέρες και 4 μήνες ο ασθενής παρέμεινε ασυμπτωματικός με σαφώς βελτιωμένη ακτινολογική εικόνα. Συμπερασματικά, ακτινολογική εικόνα ανάστροφου σημείου της άλω στην HRCT δεν αποτελεί παθογνωμονικό σημείο μίας νόσου και συνιστάται να διερευνηθούν όλα τα αίτια εμφάνισης της RHS.


Λέξεις - Κλειδιά: Ανάστροφο σημείο της άλω, υψηλής ανάλυσης αξονική τομογραφία, πνευμονίας της κοινότητας

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The Rivulet Sign

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A twenty-four-year-old man was referred to us in view of high grade fever, breathlessness, right-sided pleuritic chest pain, dry cough since 2 months and abdominal pain since 6 months. He was a chronic-alcohol-consumer. Examination revealed fever, tachycardia, tachypnea, signs of right massive pleural effusion, abdominal distension and tenderness. The blood investigations including serum amylase were normal. The pleural fluid analysis confirmed pancreatitis-associated-empyema with high pleural fluid amylase (13705U/L). Contrast-Enhanced-Computed-Tomography (CT) of thorax & abdomen was reported as acute-on-chronic pancreatitis with intra and peripancreatic, posterior mediastinal collections, minimal pericardial, right pleural effusion with a pancreatico-pleural fistula (PPF) extending through the diaphragmatic hiatus connecting the mediastinal pleura and the intrapancreatic collections. The CT-sagittal-reconstruction image demonstrated the PPF which appears like a small stream of water (figure, red arrow). Hence we it named “The Rivulet Sign”. He was managed with intercostal drainage, broad spectrum antibiotics and octreotide with resolution empyema and closure of the fistulous tract.

PPF is a complication of acute/chronic pancreatitis. It develops due to leak from an incompletely formed or ruptured pseudocyst or direct pancreatic duct leak. The duct disrupts posteriorly, pancreatic secretion flows through diaphragmatic hiatus into mediastinum/pleura forming a PPF.1 High clinical suspicion and pleural fluid amylase clinches the diagnosis. CT demonstrates the fistula in 50% and endoscopic-retrograde cholangiopancreatography (ERCP) or magnetic-resonance-cholangiopancreatography (MRCP) in 80%.2 Treatment modalities include (1) octreotide and thoracentesis, (2) ERCP with stent placement, (3) surgery.1 Our case highlights the rare complication of PPF with a novel radiologic sign “The Rivulet sign”.

Conflicts of interest. None.

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FIGURE 1. HRCT was performed showing severe bilateral cystic bronchiectasis lesions affecting all lobes, more excessive in middle lobe, lingula and lower lobes bilaterally.
Pulmonary Langerhans Cell Histiocytosis
Evolution of radiologic findings after smoking cessation

We present the case of a 30 year old male with non-productive cough for the last 2 months. No other symptoms were reported. He had been a farmer since the age of 15 and was a current smoker (1 pack of cigarettes for 25 years). His personal medical history was negative and he was on no medication. Physical examination revealed no abnormal findings. High Resolution Computed Tomography (HRCT) revealed the presence of bilateral and symmetrically distributed innumerable centrilobular nodules and cysts with clearly perceptible walls allowing them to be differentiated from emphysema. The abnormal findings had striking upper lobe predominance, with characteristic sparing of the costophrenic angles. The patient was subjected to bronchoscopy and bronchoalveolar lavage (BAL). The results of BAL were: Macrophages: 78%, Lymphocytes: 18%, Eosinophils: 1%, Neutrophils: 3%, CD1a: 6%. The combination of radiologic and BAL findings secured the diagnosis of Pulmonary Langerhans Cell Histiocytosis (PLCH) obviating the need for tissue confirmation. Smoking cessation was strongly advised. A new HRCT performed 9 months later showed an almost complete resolution of radiographic findings.

It is worth noting that the early “cavitation” of nodules seen in PLCH is due to the bronchocentric localization of inflammation and not to a necrotic process, hence the quotation marks. As the granulomatous inflammation progresses in the peribronchial area, it causes destruction of the bronchiolar wall and dilation of the lumen. The resulting increased contrast in attenuation between the bronchial wall/peribronchial area and the airway lumen gives the impression of early “cavitation”. This also explains the radiologic progression of PLCH from nodules to thick wall cysts to thin wall cysts and finally to bizarre shaped cysts.

With this case, we would like to highlight the characteristic HRCT findings of PLCH, the potential diagnostic value of BAL and also the fact that in term of management smoking cessation is of utmost importance.

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Septic thromboembolism in intravenous drug users

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- Intravenous drug users
- Septic pulmonary emboli
- Feeding vessel sign

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A 35-year-old male, intravenous drug user (IVDU), was admitted because of fever and cough with blood-tinged sputum. Chest X-ray revealed multiple pulmonary lesions (not shown). Contrast enhanced chest and abdominopelvic computed tomography (CT) demonstrated multiple pulmonary nodules with cavitation (arrowheads, Panel A) with the presence of feeding vessel sign highly suggestive but not pathognomonic of the septic nature of them (arrows, Panel A) and the relevance of extensive thrombosis with the presence of air within the thrombus, in the inferior vena cava (arrowhead, Panel B). Transthoracic ecocardiography shown vegetation at the aortic valve. Treatment was started with vancomycin plus gentamycin, and low-molecular-weight-heparin. As blood cultures subsequently grew Staphylococcus Aureus methicillin-sensitive, antimicrobial treatment continued with oxacillin for 4 weeks, and the patient had a full recovery. Drug injection into proximal veins may lead to septic deep vein thrombosis. Often septic pulmonary emboli are the first indication of a serious underlying focus of infection, either right-sided endocarditis or venous sepsis. Frequently the clinical picture is one of severe pneumonia with staphylococcal septicaemia1. CT is useful in demonstrating the full extent of thrombotic occlusion of proximal veins, recognize septic pulmonary emboli and pathologies of adjacent structures1,2.

FIGURE 1

COMPETING INTERESTS

All the authors declare that they do not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. No conflict of interest to declare.

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INSTRUCTIONS FOR AUTHORS

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All manuscripts should be accompanied by a cover letter, signed by the corresponding author, clearly stating the following:
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<table>
<thead>
<tr>
<th>Type of publication</th>
<th>Abstract (words)</th>
<th>Body of Manuscript (words)*</th>
<th>References (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Editorial</td>
<td>n/a</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>Original Research</td>
<td>250</td>
<td>3500</td>
<td>50</td>
</tr>
<tr>
<td>Reviews</td>
<td>250</td>
<td>4500</td>
<td>100</td>
</tr>
<tr>
<td>Case Reports</td>
<td>150</td>
<td>1500</td>
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</tr>
<tr>
<td>Special Articles</td>
<td>250</td>
<td>2500</td>
<td>50</td>
</tr>
<tr>
<td>Correspondence</td>
<td>n/a</td>
<td>500</td>
<td>5</td>
</tr>
</tbody>
</table>

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2. The clinical relevance of the work described and what it adds to the current literature.

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Acknowledgements
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